

Protocol: Colony PCR

(see sequencing notes)

1 uL of forward primer (10 uM)

1 uL of reverse primer (10 uM)

1 uL of template

10 uL of polymerase (TAQ→ comes in 2x master mix (polymerase, buffer and DNTPs))

7 uL of water

total: 20uL reaction

where will we get our template from?

you can do a miniprep, but that takes too long

TEMPLATE:

20 uL sterile water

1 colony picking

pipet up and down

we still need to do miniprep before sequencing

Hence, for each colony you pick, use 1 uL for the template, and 15uL to start a miniprep

Machine settings:

95 C for 5 minutes

95,55,68,4

25X